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In vitro antimycobacterial activity of six Cameroonian medicinal plants using microplate alamarBlue assay ☆

Céline Nguefeu Nkenfou [a,b,*], *Isabelle Kamga Mawabo* [a,c], *Augustin Notedji* [a], *Jean Nkenfou* [a], *Patrick Valere Tsouh Fokou* [d], *Jean Bosco Jouda* [a,e], *Jules-Roger Kuiate* [c]

[a] *Molecular Biology Center, P.O. Box 14495, Yaoundé, Cameroon*
[b] *Higher Teacher Training College (École Normale Supérieure), University of Yaoundé I, Yaoundé, Cameroon*
[c] *Department of Biochemistry, University of Dschang, Dschang, Cameroon*
[d] *Department of Biochemistry, University of Yaoundé I, Yaoundé, Cameroon*
[e] *Department of Chemistry, University of Yaoundé I, Yaoundé, Cameroon*

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ABSTRACT

Objective/background: The latest incidence of tuberculosis (TB) (per 100,000 people) in Cameroon was 243.00 as of 2011. Over the past 21 years, the value for this indicator has fluctuated between 112.00 in 1990 and 320.00 in 2003. Worldwide, this incidence has also increased, bringing back TB as a reemerging disease. On the same note, resistance to anti-TB drugs has increased, urging the search for new molecules.
Methods: This study was carried out to evaluate the antimycobacterial activity of six medicinal plants on the virulent strain, H37Rv, using the microplate alamarBlue assay. *Mycobacterium tuberculosis* (H37Rv strain) was incubated with decreased concentrations of six plant extracts, ranging from 250 μg/mL to 31.25 μg/mL. After 7 days of incubation at 37 °C, the effects of these plant extracts on the viability of the mycobacteria were evaluated. For each plant extract, the minimal inhibitory concentration was determined.
Results: The results showed that the compounds MBC1, MBC24, MBC68, MBC81, MBC117, and MBC118 were the best candidates with minimal inhibitory concentrations of 31.25, 62.5, 125, 62.5, and 125 μg/mL, respectively.
Conclusion: These results confirm and validate the traditional use of these plants to treat respiratory diseases, which could be good sources and alternatives of plant metabolites for anti-TB-drug development.
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Introduction

Tuberculosis (TB) is a global pandemic caused by *Mycobacterium tuberculosis* [1,2]. Globally, there are an estimated 9.3 million new cases and 13.7 million chronic active cases responsible for 1.7 million deaths worldwide yearly. About one-third of the world's population is at risk to develop active TB and contribute to the continued spread of *M. tuberculosis*

* Corresponding author at: Molecular Biology Center, P.O. Box 14495, Yaoundé, Cameroon.
E-mail address: nkenfou@yahoo.com (C.N. Nkenfou).
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within communities [3–5]. While TB itself has historically been of central concern of public health and infectious diseases, the last few decades have witnessed the rise of human immunodeficiency virus/AIDS, an immunosuppressive illness that amplifies the infectivity of the tubercle bacillus and catalyzes its conversion from latent to active infection [6]. Furthermore, the emergence of *M. tuberculosis* strains resistant to conventional first-line and second-line antitubercular treatment is particularly worrisome [7,8], since no new anti-TB drugs have been introduced into the market since 1967. Moreover, up to 50 million people are infected with drug-resistant forms of TB with about 500,000 cases of multidrug-resistant TB a year worldwide [9]. Even though there are currently new lead compounds being characterized for TB treatment [10,11], they are challenged by poor accessibility, high costs, long treatment regimen, and low adherence owing to the toxicity of second-line drugs. The newly commercialized drug is likely to be exhausted with the emerging resistance, emphasizing the imperative continuous search, identification, and characterization of more compounds for anti-TB drugs.

Medicinal plants have been used for centuries as nonexhaustive sources of metabolites for drug development and as an alternative remedy for treating human diseases, as they contain numerous active constituents of therapeutic value [12–15]. Rational chemistry, while essential to the development of many modern pharmaceuticals, often serves better to refine the chemical blueprints isolated from natural product screens than to devise entirely new molecular backbones. The enormous diversity of plant-derived compounds therefore makes them one of the most promising reservoirs of potentially novel anti-TB molecules. Cameroon is renowned for its rich and uncharacterized biodiversity [16], and Cameroonian medicinal plants are frequently used to treat diseases as a means of reducing reliance on expensive imported and/or chemical drugs. Such plants should be identified and screened on the basis of traditional knowledge for efficacy in the treatment of TB.

This work reports on the in vitro anti-TB evaluation of medicinal plants identified during an ethnobotanical survey carried out in Kala at the Department of "Méfou-et-Akono"/C entre Region and in Dschang at the Menoua Department/West Region. This survey investigated on medicinal plants used by traditional healers to treat cough and pulmonary diseases.

Materials and methods

Plant collection and conditioning

Plants (bark, roots, and stalk) were collected based on the information obtained from traditional healers from Kala at the Department of "Méfou-et-Akono"/Centre Region and in Dschang at the Menoua Department/West Region of Cameroon. In Cameroon, generally, most people rely on traditional healers for their primary health care. These traditional healers used plants to treat persisting cough and chest pain. They have inherited the knowledge from their parents, grandparents, and ancestors.

The collected plants were identified at the National Herbarium of Yaoundé, where voucher-specimen numbers were obtained. Figs. 1 and 2 are photographs of two of the plants used (*Garcinia preussei* and *Acanthus montanus*). The plant material was dried and ground into coarse or fine powder depending on their texture.

Preparation of plant extract

Crude extracts of plant parts were prepared as follows:

1. For hot extraction, 40 g of the powdered bark or stalk was weighed and put into the extraction thimble on the Soxhlet to which 250 mL of the solvent was added. The various solvents used were methanol, hexane, and ethyl acetate. The Soxhlet was switched on and extraction was carried out for 2 h and 30 min. The solvent was removed from the extract by evaporation on a rotavapor.
2. For cold extraction, 100 g of powdered roots or stalk was weighed and added into 500 mL of the solvent. Extraction was carried out at room temperature with frequent shaking for 48 h. The filtrates were evaporated to dryness using the rotavapor.

The obtained crude extracts were thus stored at 4 °C for subsequent work. The phytochemical screening of the plant extracts was carried out by the method described by Harborne [17].

In vitro anti-TB screening

M. tuberculosis culture preparation

The *M. tuberculosis* reference strain, H37Rv, was used. Ten milliliters of Middlebrook 7H9 broth supplemented with 10% oleic acid–albumin–dextrose–catalase and 0.2% of glycerol culture was inoculated with 0.4–0.6 mL of freezer stock of H37Rv in a 50 mL conical tube. The culture was grown to mid-log phase on the wheel at 37 °C, until OD_{600} = 0.4–0.8. Using 7H9 broth without Tween 80, the culture was diluted to OD_{600} = 0.001. This resulted in a culture with approximately 10^5 CFU/mL. Then, 100 μL of this culture was used to set up the assay plates, with each well containing 10^4 CFU.

Antimycobacterial-activity tests

The antimycobacterial activity of the plant crude extracts was tested using the microplate alamarBlue assay [18,19].

Fig. 1 – Leaves of *Garcinia preussii*.

Fig. 2 – Stalk and leaves of *Acanthus montanus*.

The susceptibility test was done in 96 microtiter plates using the alamarBlue reagent as an indicator of cellular viability. Working solutions of the tested extracts were diluted in Middlebrook 7H9 broth supplemented with oleic acid–albumin–dextrose–catalase to obtain the final sample concentrations that ranged from 250 μg/mL to 31.25 μg/mL. Isoniazid was dissolved in dimethyl sulfoxide and used as a positive control drug at 1.28 μg/mL as the starting concentration, and extracts/drug-free medium with strain suspensions were used as the negative control. One hundred microliter of 7H9 broth were added into all wells of the 96-well plate, and 100 μL of the compounds/extracts was introduced to the wells in the first row (A) and mixed thoroughly. The sample mixture (100 μL) was removed from wells of row A to perform a twofold serial dilution down the rows (B–H). The last 100 μL was discarded. Then, 100 μL of the inoculum was introduced into the corresponding wells. The final volume in each well was 200 μL. Each extract concentration was assayed in duplicate. Each microplate was then sealed with the optical sealing tape and incubated for 7 days at 37 °C in normal atmosphere. After the incubation period, 32.5 μL of alamarBlue was added to each well. The plates were then reincubated for 16–18 h at 37 °C in the dark. The experimental results were computerized using the BMG, Leicester, United Kingdom OPTIMA microplate reader at 544ex/590em for data analysis.

The minimal-inhibitory-concentration (MIC) results were presented as mean value. The lowest concentration that resulted to 90% inhibition was defined as the MIC. The MIC values determined by this method were cross-checked using the broth-dilution methods. A blue color in the well was scored as "no mycobacterial growth," and a pink color was scored as "growth occurrence" [20,21].

Results

Plants collected

Table 1 presents the identification of the six studied plants, by scientific and family names, traditional usage, and voucher-specimen number. These plants belong to the following families: Annonaceae, Vitaceae, Rubiaceae, Urticaceae, Lauraceae, and Acanthaceae.

Crude-extract preparation

Six different crude extracts were prepared with different solvents of extraction. The plant parts analyzed, the solvents used, and the yields of extraction are presented in Table 2.

Antimycobacterial activity

The antimycobacterial activity of the plant crude extracts has been evaluated on the virulent strain H37Rv at the highest concentration of 250 μg/mL. The six tested extracts, namely, *A. montanus*, *Beilschmiedia obscura*, *Cissus petiolata*, *Enantia chlorantha*, *Urera repens*, and *Garcinia preussii*, were active against *M. tuberculosis* with MICs ranging from 31.25 μg/mL to 250 μg/mL. These results are summarized in Table 3.

The most active anti-TB effect was obtained from the methanolic extract of *B. obscura* with an MIC of 31.25 μg/mL that inhibits the growth of *M. tuberculosis* at 96.2%. The methanolic extract of *A. montanus* and *U. repens* each exhibited an antimycobacterial activity with an MIC of 62.5 μg/mL and a percentage of growth inhibition of 95.06% and 98.4%, respectively.

Phytochemical screening of the plant extracts

The screens identified various compounds from the plant extracts. These compounds include phenols, sterols, saponins, flavonoids, and glycosides. These results are presented in Table 4 for each plant.

Discussion

Beilschmiedia species are known to produce many types of phytochemicals [22–24] with various biological activities. Besides, Fankam, Kuiate, and Kuete [25] worked on fruits of *B. obscura*, and found that they were highly active against a panel of Gram-negative bacteria.

Plants from the Acanthaceae family are widely used traditionally for the treatment of various ailments, such as infectious diseases [26,27]. The antimycobacterial activity observed from *Acanthus montanus* corroborated with the study of Ikezu, Ajiwe, Ilozue, and Chukwukanne [28], who worked on the leaves of *A. montanus*. They found that, in comparison with the activity of some standard antibiotics, the leaves of *A. montanus* were more active against Gram-negative and Gram-positive bacteria.

The roots of *G. preussei* displayed an MIC of 125 μg/mL on the virulent strain H37Rv. The biological activity of compounds

Table 1 – Plant name, family name, and traditional use of collected plants.

Plant code	Scientific name	Family name	Traditional usage	Voucher-specimen number
MBC1	*Enantia chlorantha*	Annonaceae	Malaria, body pains, gastrointestinal troubles, cough	28724/SRF/Cam
MBC17	*Cissus petiolata*	Vitaceae	Asthma, cough, hemorrhoids, gonorrhea	9163 SRF Cam
MBC24	*Beilschmiedia obscura*	Lauraceae	Friction on localized pains, respiratory problems	1004/SRFK
MBC68	*Urera repens*	Urticaceae	Abscess, headache, purge, asthma	7450/SRF/Cam
MBC117	*Acanthus montanus*	Acanthaceae	Fever, furuncles, cancer, ulcer, cough	2127/SRFK
MBC118	*Garcinia preussei*	Clusiaceae	Stomachaches, toothaches, chewstick, cough	19325/SRF/Cam

Table 2 – Solvents used and yield of extraction.

Plant code	Scientific name	Part used	Extraction solvent	Yield (mg/100 g)
MBC1	*Enantia chlorantha*	Bark	CH_3OH Hot extraction	1.70
MBC17	*Cissus petiolata*	Stalk	CH_3OH Hot extraction	0.24
MBC24	*Beilschmiedia obscura*	Roots	$CH_3COOC_2H_5$ Cold extraction	0.70
MBC68	*Urera repens*	Stalk	CH_3OH Hot extraction	0.66
MBC117	*Acanthus montanus*	Stalk	Hexane	0.72
MBC118	*Garcinia preussei*	Roots	Hexane/ethyl acetate 50:50	1.00

Table 3 – Antimycobacterial activity of the six plant extracts.

Plant code	Name of the plant	Part used	MIC (μg/mL)	Percentage of inhibition (%)
MBC1	*Enantia chlorantha*	Bark	250	91.9
MBC17	*Cissus petiolata*	Stalk	250	97.9
MBC24	*Beilschmiedia obscura*	Roots	31.25	96.2
MBC68	*Urera repens*	Stalk	62.5	98.4
MBC117	*Acanthus montanus*	Stalk	62.5	95.06
MBC118	*Garcinia preussei*	Roots	125	96.7

MIC = minimal inhibitory concentration.

Table 4 – Results of the phytochemical tests done on four of the six plant extracts.

	Beilschmiedia obscura	*Urera repens*	*Acanthus montanus*	*Garcinia preussei*
Phenols	+	–	–	–
Triterpenes	–	–	–	–
Sterols	+	–	+++	+++
Tannins	+	+	+	+
Saponins	–	–	–	+
Flavonoids	+++	++	+++	+++
Alkaloids	–	+	–	–
Lipids	+	+	+	+
Glycosides	+	+	++	–
Sugars	–	+	+	–

The symbol "+" means "present," while the symbol "–" means "absent".

isolated from *Garcinia* on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and Enterococcus *faecalis* has already been evaluated and shows interesting activities [29]. Kaikabo and Eloff [30] isolated two biflavonoids from *Garcinia*, and found that they were active against fast-growing and nonpathogenic *Mycobacterium smegmatis*, and had a good activity against nosocomial bacteria.

Although no antibacterial activity has yet been evaluated on *C. petiolata*, a plant belonging to the Vitaceae family, Garima, Saurabh, and Nagori [31] did an overview on the

pharmacological and therapeutic activity of *Cissus quadrangularis*, a plant from the same family. They reported that two asymmetrical tetracyclic triterpenoids and calcium were identified as its major constituents, and that it possesses antibacterial and antifungal activities [32–35]. Moreover, the phytochemical study of the aerial part of *C. quadrangularis* done by Ruskin et al. [15] showed the presence of alkaloids, tannins, and flavonoids. These wide variety of phytochemical compounds could justify the antimycobacterial activity observed with *C. petiolata*.

The investigation of *E. chlorantha* stem barks showed that they contained a large quantity of phenols, alkaloids, saponins, flavonoids, and glycosides [36,37]. In addition, several reports in the literature indicate a wide variety of pharmacological activities of this plant [38,39]. This can justify the observed antimycobacterial activity.

The stalk of *U. repens* showed an interesting activity with an MIC of 62.5 μg/mL. To the best of our knowledge, the antimycobacterial activity of this plant is being reported here for the first time. Nevertheless, according to Gindri et al. [40], the leaves of *Urera baccifera*, a plant from the same genus *Urera*, inhibited the growth of *Klebsiella pneumoniae*, a Gram-negative bacterium.

It is worth pointing out that the activities showed from the plants studied are those from crude and unpurified, thus non-concentrated, compounds. It is expected that fractionation of these crude extracts will improve the MIC observed.

Conclusion

The obtained results confirm and validate the traditional use of some of these plants, which could be good sources and alternative of metabolites for anti-TB-drug development. These encouraging results prompted us to pursue the evaluation of the most active extracts. Therefore, fractionation and further phytochemical and pharmacological studies of these plants are evidently worthy, and our group is focusing on this effort.

Conflicts of interest

The authors declare that they have no competing interests.

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REFERENCES

[1] H.M. Kenneth, C.D. Hamilton, Synergistic pandemics: confronting the global HIV and tuberculosis epidemics, Clin. Infect. Dis. 50 (2010) 67–70.
[2] K. Dheda, T. Gumbo, N.R. Gandhi, et al, Global control of tuberculosis: from extensively drug-resistant to untreatable tuberculosis, Lancet Respir. Med. 2 (2014) 4321–4338.
[3] R.M. Jasmer, P. Nahid, P.C. Hopewell, Clinical practice: latent tuberculosis infection, N. Engl. J. Med. 347 (2002) 1860–1866.
[4] World Health Organization, Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response, 1211 Geneva 27, Switzerland, World Health Organization; 2010.
[5] M. Maiga, A. Abaza, W.R. Bishai, Current tuberculosis diagnostic tools & role of urease breath test, Indian J. Med. Res. 135 (2012) 731–736.
[6] M.C.A. Kochi, Global tuberculosis incidence and mortality during 1990–2000, Bull. World Health Organ. 72 (1994) 213–220.
[7] Center for Disease Control and Prevention, Division of Tuberculosis Elimination, Core curriculum on tuberculosis: what the clinician should know, 1600 Clifton Road, Atlanta, GA, USA, Centers for Diseaeses Control and Prevention; 2003.
[8] World Health Organization, Epidemiology, global tuberculosis control: epidemiology, strategy, financing, 1211 Geneva 27, Switzerland, World Health Organization; 2009. p. 6–33.
[9] WHO, Global TB control report: epidemic levelling off, 1211 Geneva 27, Switzerland, World Health Organization; 2007.
[10] R.E. Chaisson, N.A. Martinson, Tuberculosis in Africa—combating an HIV-driven crisis, N. Engl. J. Med. 358 (2008) 1089–1092.
[11] Y.A. Skeiky, J.C. Sadoff, Advances in tuberculosis vaccine strategies, Nat. Rev. Microbiol. 4 (2006) 469–476.
[12] R. Deepa, H. Manjunatha, V. Krishna, et al, Evaluation of antimicrobial activity and antioxidant activity by electrochemical method of ethanolic extract of *Pterocarpus marsupium* Roxb bark, J. Biotechnol. Biomater. 4 (2014) 166, http://dx.doi.org/10.4172/2155-952X.1000166.
[13] D. Migliore, N.P.V. Acharya, P. Jolles, Characterization of large quantities of glutamic acid in the walls of human virulent strains of mycobacteria, C. R. Acad. Sci. Hebd. Seances Acad. Sci. D 263 (1966) 846–848.
[14] W. Paolo, J. Nosanchuk, Tuberculosis in New York City: recent lessons and a look ahead, Lancet Infect. Dis. 4 (2004) 287–293.
[15] S.R. Ruskin, K.V.M. Priya, S.T. Gopukumar, et al, Evaluation of phytochemical, antibacterial and anti-cancerous activity of *Cissus quadrangularis* from South Western Ghats regions of India, Int. J. Pharm. Sci. Rev. Res. 28 (2014) 12–15.
[16] P.J. Brennan, H. Nikaido, The envelope of mycobacteria, Annu. Rev. Biochem. 64 (1995) 29–63.
[17] J.B. Harborne, Phytochemical Methods, second ed., Chapman & Hall, London, 1973.
[18] L.A. Collins, S.G. Franzblau, Microplate alamar blue assay versus BACTEC 460 for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*, Antimicrob. Agents Chemother. 41 (1997) 1004–1009.
[19] D. Webster, T.D. Lee, J. Moore, et al, Antimycobacterial screening of traditional medicinal plants using microplate resazurin assay, Can. J. Microbiol. 56 (2010) 487–494.
[20] A. Martin, F. Portaels, J.C. Palomino, Colorimetric redox-indicator methods for the rapid detection of multidrug resistance in Mycobacterium tuberculosis: a systematic review and meta-analysis, J. Antimicrob. Chemother. 59 (2007) 175–183.
[21] T.P. Primm, S.G. Franzblau, Recent advances in methodologies for the discovery of anti-mycobacterial drugs, Curr. Bioact. Compd. 3 (2007) 1–8.
[22] N.B. Lenta, F. Tantangmo, P.K. Devkota, et al, Bioactive constituents of the stem bark of *Beilschmiedia zenkeri*, J. Nat. Prod. 72 (2009) 2130–2134.
[23] C. Tchiegang, M. Parmentier, Chemical composition and nutritional evaluation of two Cameroonian soup thickeners: *Belschmiedia jacques felexii* and *Belschmiedia anacardiodes*, Int. J. Food Sci. Technol. 45 (2008) 187–189.

[24] P.S. Yang, M.J. Cheng, I.S. Chen, Two new endiandric acid analogs, a new benzopyran and a new benzenoid from the root of *Beilschmiedia erythrophloia*, Helv. Chim. Acta 91 (2008) 2130–2138.
[25] A.G. Fankam, J.-R. Kuiate, V. Kuete, Antibacterial activities of *Beilschmiedia obscura* and six other Cameroonian medicinal plants against multidrug resistant Gram-negative phenotypes, BMC Complement Altern. Med. 14 (2014) 1–9.
[26] C.O. Okoli, P.A. Akah, N.J. Onuoha, et al, *Acanthus montanus*: an experimental evaluation of the antimicrobial, anti-inflammatory and immunological properties of a traditional remedy for furuncles, BMC Complement Altern. Med. 8 (2008) 27, http://dx.doi.org/10.1186/1472-6882-8-27.
[27] B.P. Kamga, B.V. Penlap, D. Lontsi, et al, Antibacterial activities of the extracts from leaves of *Acanthus montanus* (Nees) T. Anders (Acanthaceae), Pharmacol. Online 2 (2008) 397–403.
[28] U.J.M. Ikezu, V.I.E. Ajiwe, N.M. Ilozue, et al, Structural elucidation and antimicrobial analysis of chloroform leaf extract of *Acanthus montanus*, J. Appl. Chem. 7 (2014) 72–75.
[29] B.B. Messi, L.K. Ndjoko, A.B. Hertlein, et al, Preussianone, a new flavanone–chromone biflavonoid from *Garcinia preussii* Engl, Molecules 17 (2012) 6114–6125.
[30] A.A. Kaikabo, J.N. Eloff, Antibacterial activity of two biflavonoids from Garcinia livingstonei leaves against Mycobacterium smegmatis, J Ethnopharmacol. 138 (2011) 253–255.
[31] M. Garima, S. Saurabh, B.P. Nagori, Pharmacological and therapeutic activity of *Cissus quadrangularis*: an overview, Int. J. Pharm. Technol. Res. 2 (2010) 1298–1310.
[32] M. Jainu, C.S. Devi, Effect of *Cissus quadrangularis* on gastric mucosal defensive factors in experimentally induced gastric ulcer—a comparative study with sucralfate, J. Med. Food 7 (2004) 372–376.
[33] O.C. Enechi, I. Odonwodo, An assessment of the phytochemical and nutrient composition of the pulverized root of *Cissus quadrangularis*, Bio-Research 1 (2003) 63–68.
[34] M. Mehta, N. Kaur, K.K. Bhutani, Determination of marker constituents from *Cissus quadrangularis* Linn. and their quantitation by HPTLC and HPLC, Phytochem. Anal. 12 (2001) 91–95.
[35] D.A. Shirley, S.P. Sen, High-resolution X-ray photoemission studies on the active constituents of *Cissus quadrangularis*, Curr. Sci. 35 (1966) 317.
[36] A.A. Adesokan, M.A. Akanji, M.T. Yakubu, Antibacterial potentials of aqueous extract of *Enantia chlorantha* stem bark, Afr. J. Biotechnol. 6 (2007) 2502–2505.
[37] O.E. Adebiyi, M.O. Abatan, Phytochemical and acute toxicity of ethanolic extract of *Enantia chlorantha* (oliv) stem bark in albino rats, Interdiscip. Toxicol. 6 (2013) 145–151.
[38] E.O. Agbaje, A.O. Onabanjo, Antimalarial properties of *Enantia chlorantha*, Ann. Trop. Med. Parasitol. 85 (1991) 585–590.
[39] R.F. Atata, A. Sanni, S.M. Ajewole, Effect of stem bark extracts of *Enantia chlorantha* on some clinical isolates, Biokemistri 15 (2003) 84–92.
[40] A.L. Gindri, T.F. Kubiça, D.N. Mario, et al, Antiviral, antimicrobial and anti-inflammatory activities of *Urera baccifera* (L.) Gaudich, Afr. J. Pharm. Pharmacol. 8 (2014) 284–291.